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Characterization of Soluble Microbial Products as Precursors of Disinfection Byproducts in Drinking Water Supply

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Abstract

Water pollution by wastewater discharge can cause the problem of disinfection byproducts (DBPs) in drinking water supply. In this study, DBP formation characteristics of soluble microbial products (SMPs) as the main products of wastewater organic biodegradation were investigated. The results show SMPs can act as DBP precursors in simulated wastewater biodegradation process. Under the experimental conditions, stabilized SMPs had DBPFP (DBP formation potential) yield of around 5.6 μmol $mmol^{-1}$-DOC (dissolved organic carbon) and DBP speciation profile different from that of the conventional precursor, natural organic matter (NOM). SMPs contained polysaccharides, proteins, and humic-like substances, and

the latter two groups can act as reactive DBP precursors. SMP fraction with molecular weight <1 kDa accounted for 85% of the organic carbon and 65% of the DBP formation. As small SMP molecules are more difficult to remove by conventional water treatment processes, more efforts are needed to control wastewater-derived DBP problem in water resource management.

Keywords: disinfection byproducts (DBPs), soluble microbial products (SMPs), DBP precursors, wastewater discharge, water pollution

1 Introduction

The formation of disinfection byproducts (DBPs) in drinking water has been a major problem of drinking water quality with its considerable public health concerns. The main organic DBP groups formed by chlorination include trihalomethanes (THMs) and haloacetic acids (HAAs). It has been found that THMs, many HAA species such as dichloroacetic acid (DCAA), and certain other halogenated DBPs are carcinogenic, mutagenic, or teratogenic (Legay et al., 2010). Natural organic matter (NOM), which is mainly composed of humic substances, is known to be the major DBP precursor in surface water (Chang et al., 2001; Hua and Reckhow, 2007). Numerous studies have been conducted on NOM to determine its characteristics and DBP yield during water disinfection (Xie, 2003).

The presence of DBP precursors in source water can also be attributed to water pollution caused by human activities. Water pollution is becoming one of the most serious global environmental issues, especially in developing countries that are experiencing rapid population and economic growth. Many surface water bodies, such as rivers and lakes, are used for both wastewater disposal and fresh water withdrawal for municipal use. Upper

reaches of many water sources have been polluted by wastewater discharge (Zheng et al., 2007). Such pollution of the raw water source can greatly affect the drinking water quality. Researchers have found that wastewater organic pollutants increase DBP precursors in the receiving water, resulting in more DBP formation in the finished drinking water (Chen et al., 2009; Chu et al., 2002; Krasner et al., 2009). There is thus a need to address the problem of wastewater-derived DBPs in water supply.

Wastewater organics are mainly composed of biodegradable compounds that would undergo biodegradation after being discharged into the upstream of receiving water sources (Dignac et al., 2001; Dignac et al., 2000). Soluble microbial products (SMPs) are an important result of the biological degradation process (Aquino and Stuckey, 2002; Liu and Li, 2010). SMPs are organic compounds that are released by microorganisms into solution from substrate metabolism and microbial cell decay. SMPs can be grouped into two categories based on the microbial growth phases: utilization-associated products (UAPs), which are SMPs that are associated with substrate metabolism and biomass growth, and biomass-associated products (BAPs), which are SMPs resulted from the biomass decay (Barker and Stuckey, 1999). The exact chemical compositions for UAP- and BAP-based SMPs remain to be determined. However, conceptually, UAPs are produced in the early phase of organic biodegradation and BAPs are released in the later phase of biodegradation when the substrates have been exhausted. According to Aquino and Stuckey (2004a), UAPs have both biodegradable and non-biodegradable fractions, while BAPs mainly contain non-biodegradable compounds.

It has been suggested that SMPs can increase the DBP formation in chlorinated water (Chen et al., 2008; Cheng and Chi, 2003; Dotson et al., 2009). The influence of SMPs on the formation of DBPs in the wastewater effluent after wastewater disinfection has been reported (Wei et al., 2011; Zhang et al., 2009). However, there are few studies on the characteristics of

SMPs as DBP precursors in the surface water supply. Moreover, the UAPs and BAPs may behave differently in DBP formation owing to their different chemical features (Boero et al., 1996). This laboratory study was conducted to investigate the properties of SMPs as DBP precursors derived from wastewater organic biodegradation in a polluted raw water source, including the dynamics of SMP production and DBP formation in biodegradation process, DBP-forming organic components, and molecular size-based DBP yield and speciation.

2 Materials and Methods

2.1 The dynamics of organic degradation, SMP production, and its DBP formation potential

Laboratory experiments were conducted to simulate the biodegradation of wastewater organics and the production of SMPs in natural water. The biodegradation experiments were carried out on glucose-based synthetic wastewater in 10-L bioreactors placed in a temperature-controlled biochemical oxygen demand (BOD) incubator at 20 °C. Glucose was selected to represent biodegradable wastewater organics, as it can be biodegraded completely leaving only SMPs as the remaining organics in the solution. Glucose (Unichem) was dissolved in Milli-Q water (Millipore) at 200 mg L^{-1}, giving a dissolved organic carbon (DOC) concentration of 80 mg L^{-1}. Activated sludge was dosed as the seed biomass at a suspended solid (SS) concentration of 2 mg L^{-1} into the bioreactors to initiate biodegradation. NH_4Cl, $FeCl_3$, $CaCl_2$, and $MgSO_4$ were added as nutrients according to the guidelines for running BOD tests (Velp Scientifica). The water pH was maintained at around 7 with a phosphate buffer solution. The solution was aerated by an air pump with an air flow rate of 4 L min^{-1} to provide oxygen to the solution. The biodegradation experiment was conducted for 15 days to study the dynamics of SMP production and DBP formation. Water samples were collected in triplicates from the incubated bioreactor every 12 h for the first 5 days and every 24 h for the next 10 days for subsequent organic analysis and DBP formation tests. The

samples were filtered through a 0.45-μm membrane (Millipore) to remove all of the bacteria and suspended solids. The filtered water, or the SMP solution, was then chlorinated to determine the DBP formation potential (DBPFP) of the dissolved organic matter in the solution after various incubation periods. The filtered water samples were also analyzed for their DOC content and UV absorbance value at 254 nm (UV_{254}).

2.2 Organic components of SMPs in relation to DBP formation

For further characterization of SMPs as DBP precursors, including the organic components and molecular weight distribution of SMPs, a more concentrated glucose solution was used for incubation to produce a sufficient amount of SMPs. The initial glucose concentration was 2000 mg L^{-1}, and the seed activated sludge concentration was 20 mg L^{-1} accordingly in the bioreactor. As described in Section 2.1, nutrients were added, the water pH was buffered at about 7, and the organic solution was aerated in the bioreactor. The aerobic incubation was carried out at 20 °C for 5 days, and the water solution was sampled every day. Samples were taken from the suspension in triplicates and filtered through a 0.45-μm membrane immediately after sampling.

The filtered samples were analyzed for their SMP contents, including carbohydrates or polysaccharides, proteins, and humic-like substances. Proteins and humic-like substances were analyzed based on the colormetric absorbance measurement with a UV/VIS spectrophotometer (UV/VIS Lambda 25, Perkin Elmer) at 750 nm following the modified Lowry method (Li and Yang, 2007) using bovine serum albumin (Sigma) and humic acid (Fluka) respectively as the standards. The carbohydrates were measured with the UV/VIS spectrophotometer at 620 nm following the phenol-sulfuric acid method (Li and Yang, 2007), with glucose as the standard.

A fluorescence spectrophotometer (Hitachi F-7000) was used to analyze the soluble organic compounds in the water samples after different degradation periods, and the results were described with fluorescence excitation-emission matrix (FEEM) (Chen et al., 2003). The spectrophotometer used a xenon excitation source, and the slits were set to 2.5 nm for both excitation and emission. To obtain the FEEMs, the excitation wavelength was increased from 220 to 400 nm in 5-nm steps. For each excitation wavelength, the emission was detected from 300 to 550 nm in 2-nm steps. Spectral subtraction was applied to the FEEM measurement to eliminate the blank spectra resulting from pure water (Her et al., 2003), and the instrumental correction was made internally following the procedure (Hitachi F-7000 Instruction Manual) recommended by the manufacturer.

2.3 Molecular weight distribution of SMPs in relation to DBP formation

The SMP samples obtained through the procedure in Section 2.2 after 5 days of biodegradation were further separated by ultrafiltration (UF) into different fractions based on molecular weight cutoffs. Ultrafiltration was carried out using a pressurized UF unit (Amicon, Millipore) as described by Aiken (1984). For the UF-based fractionation, 500 mL of the filtered water sample was passed through four membranes (Amicon, Millipore) with different cutoffs in the following sequence: 100, 30, 10, and 1 kDa. The staged filtration resulted in 5 solutions of the same volume (100 mL) with apparent organic sizes of <0.45 μm, <100 kDa, <30 kDa, <10 kDa, and <1 kDa. After the UF separation, the solutions were analyzed for their organic contents and tested for DBPFPs. Accordingly, the DOC, UV_{254}, and DBPFP of the SMP fractions within the apparent molecular weight ranges of >100 kDa, 30-100 kDa, 10-30 kDa, 1-10 kDa, and <1 kDa were determined.

2.4 DBPFP determination

DBPFP tests were conducted to evaluate the quantity and reactivity of the organic DBP precursors in the water samples. The DBP formation tests were carried out by chlorinating the filtered water samples in accordance with the Standard Methods (APHA, 2005). For each DBPFP test, a 100-mL water sample was chlorinated with NaOCl (Unichem), and the resulting solution was incubated in dark for 7 days at pH 7.0±0.2 with a 0.5 N phosphate buffer. To ensure the presence of free chlorine residue after 7 days of incubation, chlorine demand tests were conducted, i.e., the water samples were dosed with 100 mg L^{-1} Cl_2, incubated for 12 hours, and the free chlorine residue was measured. The NaOCl dose for the DBPFP test that would result in a free chlorine residue of between 3 and 5 mg L^{-1} was then determined. The actual free chlorine residue in the chlorinated water after 7 days of incubation was also measured, and only the samples that had residual free chlorine concentrations of 3-5 mg L^{-1} were used. Immediately after the incubation, the excess chlorine in the water samples was quenched with NH_4Cl (BDH), and the DBP compounds formed in the chlorinated solution were extracted.

The following main groups of organic DBPs were detected: THMs and HAAs (the most predominant and commonly regulated DBP groups), trihaloacetaldehydes (the third largest group of organic DBPs in chlorinated water), halopropanones (commonly detected in chlorinated water after the previous three groups), and nitrogenous DBPs (N-DBPs) including haloacetonitriles and trihalonitromethanes (at lower concentrations but imposing higher health risks).

The methods for the liquid-liquid extraction and chemical analysis for the volatile DBP groups, including THMs, trihaloacetaldehydes, halopropanones, and N-DBPs, were developed according to EPA Method 551.1 (USEPA, 1995). Methyl tert-butyl ether (MTBE, BDH) was used as the solvent for the liquid-liquid extraction. The methods for HAA extraction and chemical analysis were developed based on EPA Method 552.3 (USEPA,

2003). HAAs in the water samples were extracted by liquid-liquid extraction with MTBE. Derivatization was then performed on the extract by adding acidic methanol at a 1:1 (vol/vol) ratio. The DBP species were analyzed with an HP 6890 gas chromatograph (GC) coupled with an HP electron capture detector (ECD) (Agilent). The GC system was equipped with a DB-35MS capillary column (Agilent) with a configuration of 30 m × 0.32 mm and a film thickness of 0.25 µm. An HP 6890 Series automatic liquid sampler was used for the sample injection, and an HP GC ChemStation was used for the data processing. The detailed instrumental conditions (e.g. the GC temperature program) and the detection limits of individual DBP compounds can be found in Supplementary Material.

2.5 Analytical methods

As an indication of the biomass content, the SS concentration in each bioreactor was measured in accordance with the Standard Methods (APHA, 2005). The UV_{254} and DOC of the organic content were measured for each water sample after filtration. UV_{254} indicates the UV absorbance at 254 nm of the organic compounds in a water sample and is believed to be closely related to the DBPFP of the water (Xie, 2003). A UV-visible spectrophotometer (UV/VIS Lambda 25, Perkin Elmer) with a 1-cm cuvette cell was used to determine the UV_{254}. The DOC was determined by a total organic carbon (TOC) analyzer (IL550, Lachat) based on the catalytic combustion-infrared method. The specific UV absorbance (SUVA) of the organic matter in water was determined by dividing the UV_{254} by the DOC concentration. Similarly, the DBPFP yield of the organic matter in the water sample was determined by dividing the DBPFP value by the DOC.

2.6 QA/QC

The experiments on the dynamics of organic degradation and SMP production in relation to DBP formation, organic components of SMPs, and molecular weight distribution of SMPs were repeated at least three times to ensure the reproducibility and reliability of the results. A set of typical experimental results is reported in the Results section, and more results of the replicated experiments on organic degradation and related DBP formation are provided as Supplementary Material (Figs. S1 and S2). During organic biodegradation incubation, triplicate samples have been taken from the bioreactor for the following experiments of dynamics of SMP production and DBP formation, organic components of SMPs, and molecular weight distribution of SMPs. Blank water samples from the bioreactors without the biomass and substrate additions were also processed with chlorination to determine the background DBP formation potentials. For DBP analysis, a calibration curve was processed with each batch of samples to ensure the accuracy of the DBP quantitation. One procedure blank was analyzed with each batch of samples to detect the background level. An internal standard, 1,2,3-trichloropropane (Sigma), was also used to examine the stability of the GC measurement.

3 Results and Discussion

3.1 The dynamics of organic degradation, SMP production, and its DBP formation potential

The simulated wastewater organic biodegradation process contained two phases. During the first organic utilization and biomass growth phase starting from the initial condition, after a one-day lag time, the DOC in the suspension decreased rapidly in less than two days from around 80 to 7 mg L^{-1}, while the SS concentration increased from 2 to around 70 mg L^{-1}, indicating great biomass growth (Fig. 1). From day 3 when the feed organic carbon was completely depleted, the biomass content began to decrease due to decay, and the SS decreased to about 17 mg L^{-1} until day 15. During this organic depletion and biomass decay

phase, the DOC in the solution remained almost constant, around 5 mg L^{-1} (Fig. 1). As glucose is a highly biodegradable organic substrate, the organic residue in the suspension after three days can be attributed to SMPs produced during the biodegradation process. The UV_{254} value of the water increased from non-detected to 0.033 cm^{-1} during the rapid glucose degradation (Fig. 1). UV_{254} is an indication of the UV-absorbing structure in organic molecules and is related to DBP formation (Xie, 2003). The initial glucose solution had no UV_{254} absorbance value. The increase in UV_{254} suggests the possible release of DBP-forming compounds, such as SMPs, during the biological organic degradation. With further biodegradation, the UV_{254} value of the water then decreased to 0.021 cm^{-1} during the biomass growth phase. Thereafter in the biomass decay phase, the UV absorbance increased to 0.025 cm^{-1}, probably due to the accumulation of endogenous decay products in the suspension.

DBP formation behavior of the solution during the organic biodegradation process also showed two stages (Fig. 2a). Before biodegradation, the glucose solution had a low initial DBPFP of 0.3 μmol L^{-1} for all of the DBP compounds detected. During the organic utilization and biomass growth phase, the water exhibited a dramatic change in DBPFP. There was a slight DBPFP increase to about 1.0 μmol L^{-1} after the first day of biodegradation, but on the next day the DBPFP of the organic solution increased dramatically to around 10.2 μmol L^{-1}. The significant DBPFP increase within a short period coincided with the rapid organic utilization and biomass growth observed at the same time. However, the DBPFP did not stay at this high level, but dropped to lower than 2.0 μmol L^{-1} on the following day. Then during the organic depletion and biomass decay phase, the DBPFP of the solution was much stable and showed only a slow and slight increase to a level of around 2.0 μmol L^{-1}.

The dynamic change in DBPFP of the water during organic biodegradation process is related with the bioactivity in the solution. During the first phase, the dramatic increase in DBPFP during the early days of bio-incubation correlated well with the rapid substrate

metabolism and biomass growth. The biodegradation transformed the feeding glucose into more reactive intermediate products, such as pyruvic acid and lactic acid (Bender, 2007), which have a high reactivity with chlorine (Kim and Yu, 2007; Weber et al., 2005). Given the intensive organic utilization, the DBP precursors may also be attributable to SMPs associated with substrate utilization, such as UAPs. The accumulation of the intermediate products, together with the UAP production, led to the high DBPFP level on day 2. Organic intermediates and UAPs are unstable and biodegradable, and thus underwent further biodegradation. The reduction of these two kinds of organic components resulted in a sharp DBPFP decrease. In the later phase, after day 3 when the glucose and its degradation intermediates had been largely consumed, the biomass began to undergo endogenous decay. The biomass associated SMPs, such as BAPs, then became the main DBP precursors. These results suggest that although UAPs and organic intermediate products that have a strong reactivity with chlorine may bring about an extremely high level of DBP formation, this effect however is temporary and diminishes with organic degradation. SMPs in the form of BAPs then remain as more stable DBP precursors.

The DBP species formed upon chlorination include chloroform (CF) for THMs, DCAA and trichloroacetic acid (TCAA) for HAAs, chloral hydrate (CH) for trihaloacetaldehydes, trichloropropanone (TCP) for halopropanones, dichloroacetonitrile (DCAN) for haloacetonitriles, and trichloronitromethane (TCNM) for trihalonitromethanes (Fig. 2). As bromide was not included in the feed substrates, no brominated-DBPs were detected in the study. DCAN and TCNM are both nitrogenous DBPs. As there is no nitrogen in glucose, the formation of N-DBPs can be attributed to nitrogenous SMPs, such as amino acids and proteins, produced during organic metabolism (Barker and Stuckey, 1999). It has been reported that nitrogenous DBPs are more toxic than regulated DBPs such as THMs and HAAs (Muellner et al., 2007).

Chloroform was the most abundant DBP species throughout the biological organic degradation process (Fig. 2b). The CH formation increased considerably, especially during the organic utilization phase. This potential may have derived from the precursors of simple and small molecules produced as intermediate products during glucose degradation, such as pyruvic acid and lactic acid (Bender, 2007). These intermediate products have oxygen-rich and biodegradable groups, such as hydroxyl, carboxyl, and carbonyl groups, that are rather reactive to chlorine (Kim and Yu, 2007; Weber et al., 2005). The CH fraction initially exceeded that of HAAs. However, with the degradation of the biodegradable CH precursors, the proportion of CH in the DBPs decreased, whereas the proportion of HAAs increased in the later phase of incubation (Fig. 2b). The HAA precursors were apparently more recalcitrant to biodegradation than the CH precursors. Of the two HAA species, a higher amount of DCAA was produced from the solution in the early phase of biodegradation (Fig. 2a). In the later phase, DCAA formation somewhat decreased, whereas the TCAA formation potential remained largely unchanged. This comparison suggests that TCAA comes from more refractory organic precursors, and DCAA is likely to be formed from both biodegradable and refractory organic molecules (Hua and Reckhow, 2007).

All of the organic residues in the solution were SMPs following the organic depletion after day 3 of the biodegradation process. Under the experimental conditions, the dissolved organics in the SMP solutions had an averaged SUVA of 0.06 L cm^{-1} $mmol^{-1}$. The average DBPFP yield of the SMPs was 5.6 μmol $mmol^{-1}$-DOC. For the chlorinated DBPs formed by SMPs, THMs (CF) were the dominant DBP species, followed by HAAs and then CH, and N-DBPs were found at a trace level. There was more DCAA than TCAA formed from the SMPs after chlorination, which is distinct from the typical speciation profile of DBPs formed by NOM, which yields much more TCAA than DCAA (Hua and Reckhow, 2007). The N-DBP fraction of the DBPs formed from the SMPs was also higher than that of the DBPs from

humic substances (Liu and Li, 2010), probably due to the higher organic nitrogen content of SMPs (Dotson et al., 2009).

3.2 Organic components of SMPs in relation to DBP formation

To further characterize the SMPs as DBP precursors, a more concentrated glucose solution was used to produce SMPs at a higher level. The incubation for SMP production lasted for five days. Similar to the organic degradation process previously described, the feeding glucose was degraded almost completely after approximately 2 days. Afterwards, biomass would undergo endogenous decay. The solution was analyzed each day for the contents of carbohydrates, proteins, and humic-like substances (Fig. 3). The initially high carbohydrate content is attributable to the feeding glucose. When glucose was degraded, the carbohydrate content dropped to 10.8 mg L^{-1}. During the glucose degradation, proteins and humic-like substances were produced as SMPs (i.e. UAPs) in the solution. Compared with carbohydrates, proteins and humic-like substances are more active DBP precursors (Xie, 2003). Moreover, proteins with organic nitrogen content can be precursors of the more harmful N-DBPs (Muellner et al., 2007). Then on the second day of biodegradation, content of humic-like substances reduced, while that of proteins had no obvious change. After day 2, the concentrations of carbohydrates, proteins, and humic-like substances kept increasing as a result of the accumulation of the biomass decay products (i.e. BAPs). By day 5, the SMP solution contained carbohydrates at 20.2 mg L^{-1}, proteins at 8.2 mg L^{-1}, and humic-like substances at 20.8 mg L^{-1}. Compared with the more conventional DBP precursor, NOM, that are normally dominated by humic substances, SMPs have higher carbohydrate and protein contents.

FEEM spectra were obtained for further analysis of the chemical features of the SMP compounds produced during organic degradation. For the pure glucose solution, there was

little spectral data detected in the FEEM spectrum. During the biomass growth stage, on the first day, when UAPs were the predominant SMPs, three groups of organic substances, including proteins, polycarboxylate-type humic acid, and polyaromatic-type humic acid, could be identified from the FEEM (Fig. 4). These three groups of organics, especially polyaromatic-type humic acid, can be reactive DBP precursors (Xie, 2003). Lai et al. (2007) also used FEEM to study the SMP composition and identified two main peaks for protein- and humic-like organics. During the organic degradation, the protein content continued to increase on day 2 in the biomass growth phase. However, the response for polyaromatic-type humic acid disappeared from the FEEM, and the response for polycarboxylate-type humic acid also decreased. Then during the biomass decay stage after day 2, when BAPs became more dominant SMPs in the suspension, the responses of both polycarboxylate-type humic acid and proteins kept increasing. The results indicate that UAPs are more biodegradable than BAPs. UAPs can be produced during the organic utilization phase and then probably undergo biodegradation. The FEEM comparison between different samples also suggests different chemical compositions of UAPs and BAPs, as UAPs (day 1 sample) appeared to contain polyaromatic-type humic acid which were not found for BAPs (day 3 or later samples).

3.3 Molecular weight distribution of SMPs in relation to DBP formation

Ultrafiltration was applied to separate the SMPs (day 5 sample) into organic fractions with different molecular sizes (Fig. 5a). The results indicate that the SMP organics were dominated by small molecules, as the fraction of <1 kDa had a DOC of 20.2 mg L^{-1}, which accounted for about 85% in weight of the organics in the SMP solution. Other researchers have also reported that small molecules predominate in SMPs (Aquino and Stuckey, 2002; Aquino and Stuckey, 2004b). The fraction of small organics with a molecular cutoff of <1 kDa had a DBPFP of up to 7.3 μmol L^{-1}, which contributed to around 65% of the total

DBPFP of the SMP solution. However, the DBPFP yield of the small organic molecules was generally lower than that of the large molecules, implying a lower reactivity of the small SMPs with chlorine in forming DBPs. Moreover, the small SMP molecules had lower SUVA values than the large SMP molecules (Fig. 5b).

The DBPs formed by SMPs smaller than 1 kDa had more CH formation (Fig. 6). In comparison, the SMP fractions of the larger molecules had more CF and DCAA production. The proportion of TCAA formation was comparable across the various SMP fractions. The results indicate that small SMPs are likely to form more CH, whereas large SMPs tend to produce more CF and DCAA, and the molecular size has little effect on the fraction of TCAA. In general, small SMP molecules were the main group of DBP precursors with a high CH fraction, whereas large-sized SMPs had a higher DBP yield of CF and DCAA formation.

Compared with NOM, which is the conventional DBP precursor in natural source water (Hua and Reckhow, 2007), SMPs appear to be composed of a higher portion of smaller molecules. In drinking water treatment, enhanced coagulation and flocculation have been put forward as the main methods of DBP control, as enhanced coagulation can selectively and effectively remove large and hydrophobic NOM molecules (Soh et al., 2008; Volk et al., 2000). However, the removal by enhanced coagulation of the small molecules of SMPs from a polluted water supply is likely to be rather limited, and they will largely pass through the water treatment processes and result in SMP-derived DBP problems with drinking water. SMPs as DBP precursors should not be overlooked in water supply and water resource management. The efficiency of current water treatment systems for removing SMP-based DBP precursors from polluted raw water should be evaluated. There is also a need for advanced treatment technologies for more effective control of wastewater-derived DBP problems.

4 Conclusions

Organic degradation in water produces SMPs which can be DBP precursors. The stabilized SMPs had DBPFP yield of around 5.6 μmol $mmol^{-1}$-DOC with a DBP speciation profile different from that of NOM. SMPs contained carbohydrates, proteins, and humic-like substances, and the latter two groups were more reactive in DBP forming. Small molecules with molecular weight <1 kDa prevailed in the SMP solutions, accounting for 85% of the DOC and 65% of the DBPFP. As small SMP molecules are more difficult to remove by conventional water treatment processes, more efforts are needed to control wastewater-derived DBP problems in water resources management.

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Figure captions:

Fig. 1. DOC, biomass concentration, and UV_{254} absorbance of the organic solution during the biodegradation test.

Fig. 2. (a) DBPFP and (b) DBP species fraction of the solution during organic biodegradation.

Fig. 3. Carbohydrate, protein, and humic-like substance contents of the organic matter in the SMP solution during organic biodegradation.

Fig. 4. FEEM of the organic matter in the SMP solution during organic biodegradation (① proteins, ② polycarboxylate-type humic acid, ③ polyaromatic-type humic acid).

Fig. 5. (a) DOC and DBPFP and (b) SUVA and DBPFP yield of the SMP fractions of different molecular sizes from the day 5 sample.

Fig. 6. Fraction of DBP species formed by SMP fractions of different molecular sizes for the day 5 sample.

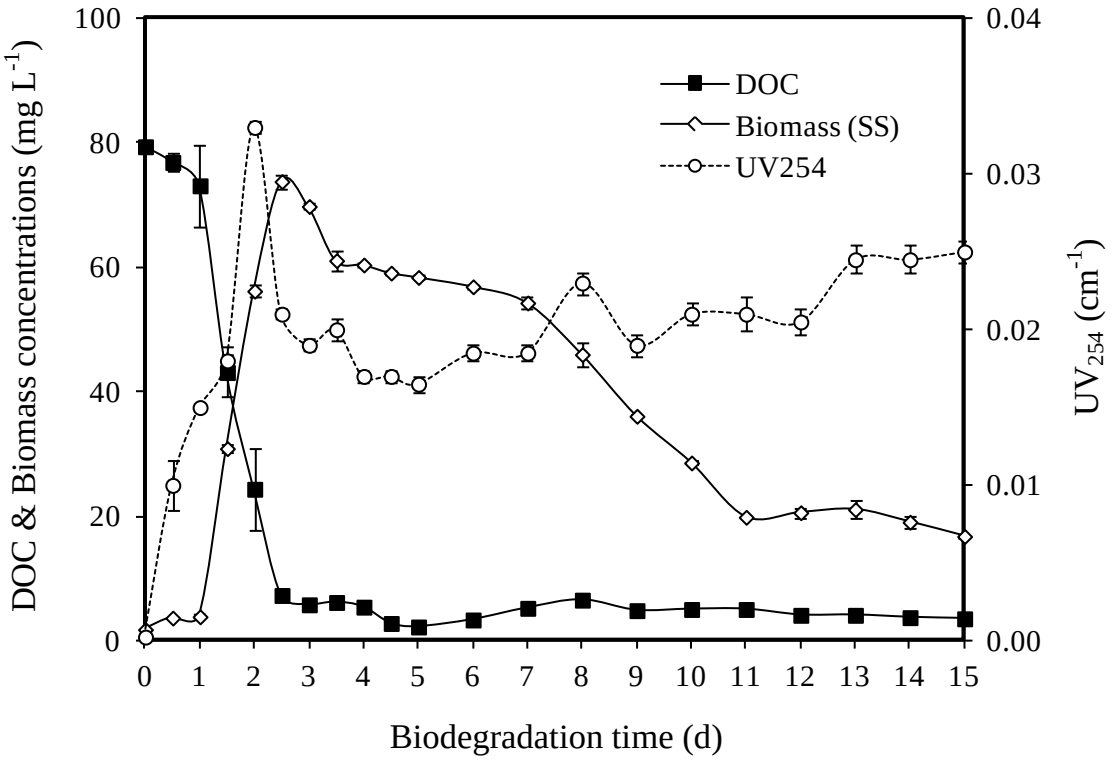


Fig. 1. DOC, biomass concentration, and UV_{254} absorbance of the organic solution during the biodegradation test.

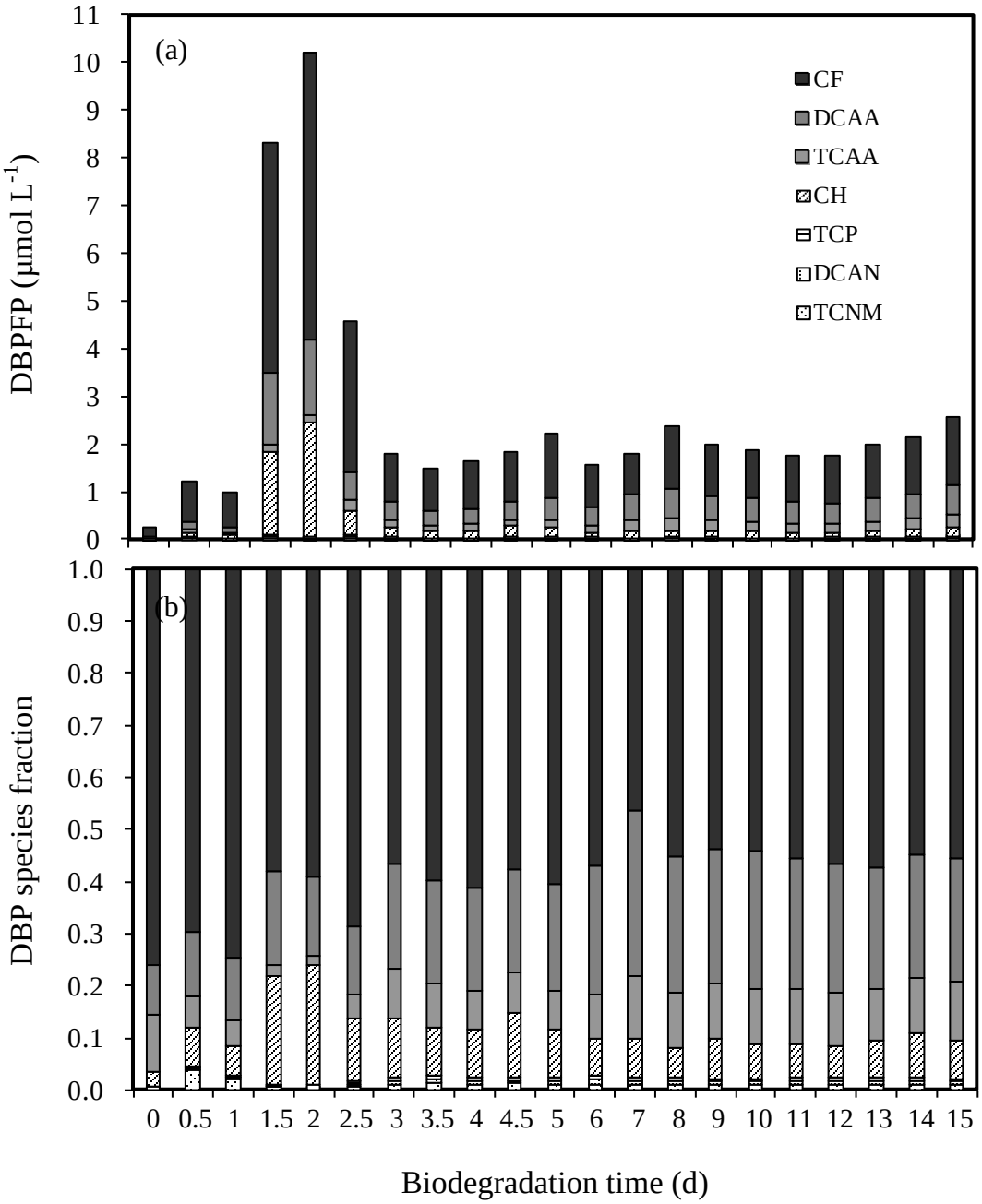


Fig. 2. (a) DBPFP and (b) DBP species fraction of the solution during organic biodegradation.

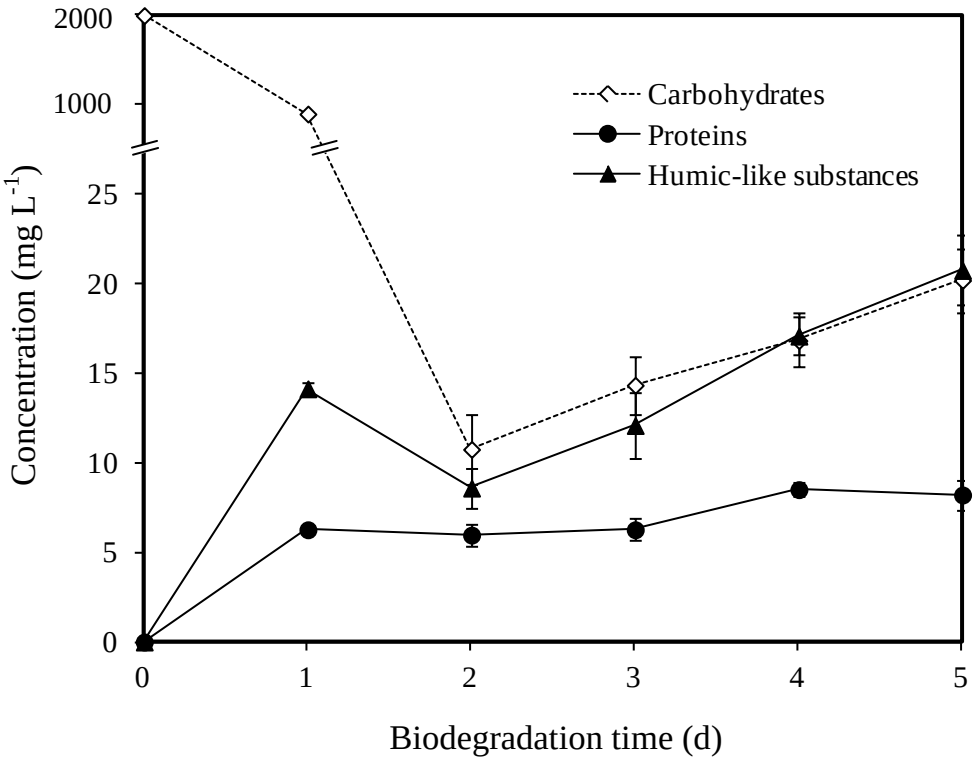
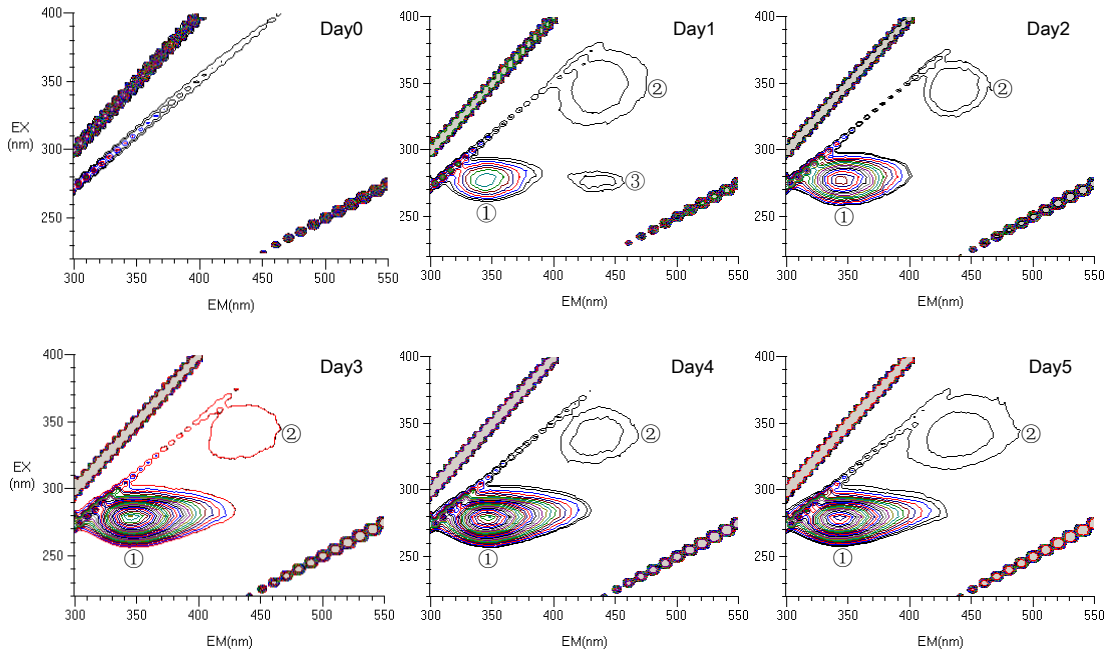


Fig. 3. Carbohydrate, protein, and humic-like substance contents of the organic matter in the SMP solution during organic biodegradation.



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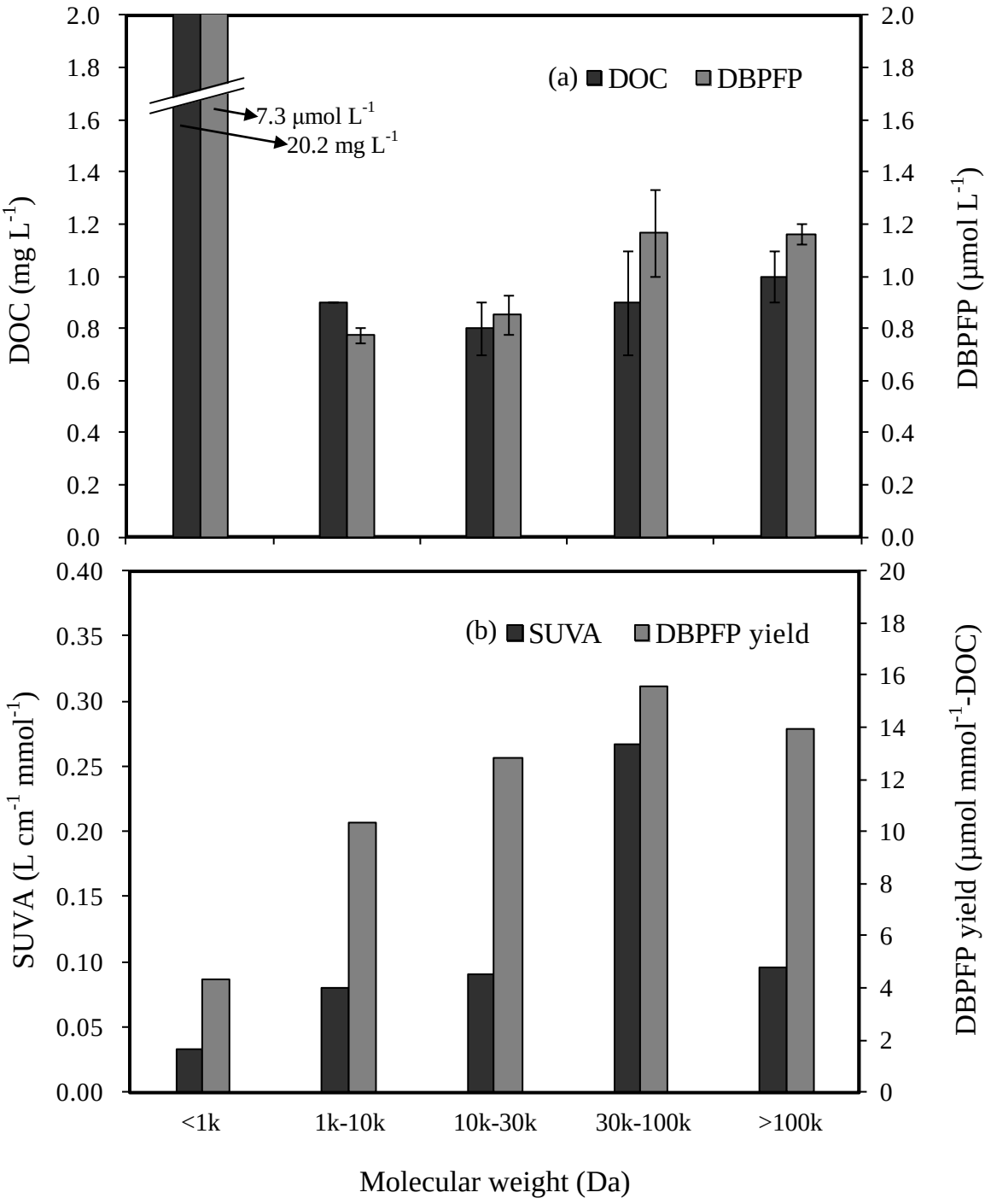

Fig. 5. (a) DOC and DBPFP and (b) SUVA and DBPFP yield of the SMP fractions of different molecular sizes from the day 5 sample.

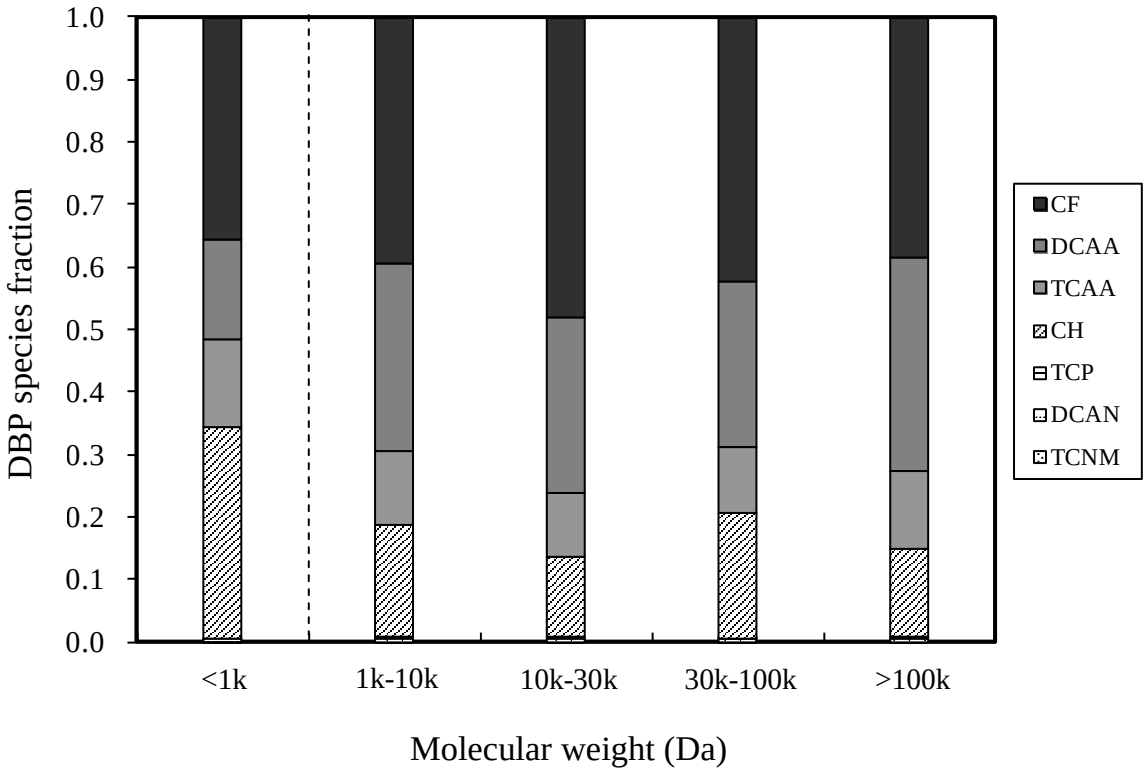

Fig. 6. Fraction of DBP species formed by SMP fractions of different molecular sizes for the day 5 sample.

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Supplementary Material

for

Characterization of Soluble Microbial Products as Precursors of Disinfection Byproducts in Drinking Water Supply

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The supplementary materials include:

1. Instrumental conditions for DBP measurement by gas chromatography (GC)

For the detection of trihalomethanes, trihaloacetaldehydes, halopropanones, haloacetonitriles, and trihalonitromethanes, one μL of the pretreated sample was introduced into the GC by splitless injection at 200 ºC. The carrier gas was N_2 at a constant flow rate of 0.8 mL min^{-1}. The initial oven temperature was set at 35 ºC and held for 9 min. The temperature was gradually increased first to 40 ºC at a rate of 2 ºC min^{-1}, then to 80 ºC at 20 ºC min^{-1}, then to 160 ºC at 40 ºC min^{-1}, where it was held for 4 min, and finally to 200 ºC, where it was held for 2 min. The detector temperature was set at 290 ºC to detect the volatile DBP groups.

For the detection of haloacetic acids, one μL of the sample was introduced into the GC by splitless injection at 200 ºC. The carrier N_2 gas was maintained at a flow rate of 0.9 mL min^{-1}. The temperature program began at 35 ºC for 10 min and increased at a rate of 5 ºC min^{-1} to 70 ºC, where it was held for 10 min, then to 120 ºC at 5 ºC min^{-1} and held for 5 min, to 135 ºC at 5 ºC min^{-1} and held for 10 min, and finally to 170 ºC at 5 ºC min^{-1}, where it was held for 5 min. The detector temperature for the HAA analysis was 260 ºC.

2. Recovery and detection limits of the DBP compounds

The recovery of studied trihalomethanes, haloacetic acids, trihaloacetaldehydes, halopropanones, haloacetonitriles, and trihalonitromethanes range from 79.0% to 97.3%. The method detection limits of most studied DBP individuals are 1 μg L^{-1}, except for chloroform whose method detection limit is 5 μg L^{-1}. Chloroform has a procedure blank of 5 μg L^{-1}. The concentration below 5 μg L^{-1} can’t be detected accurately.

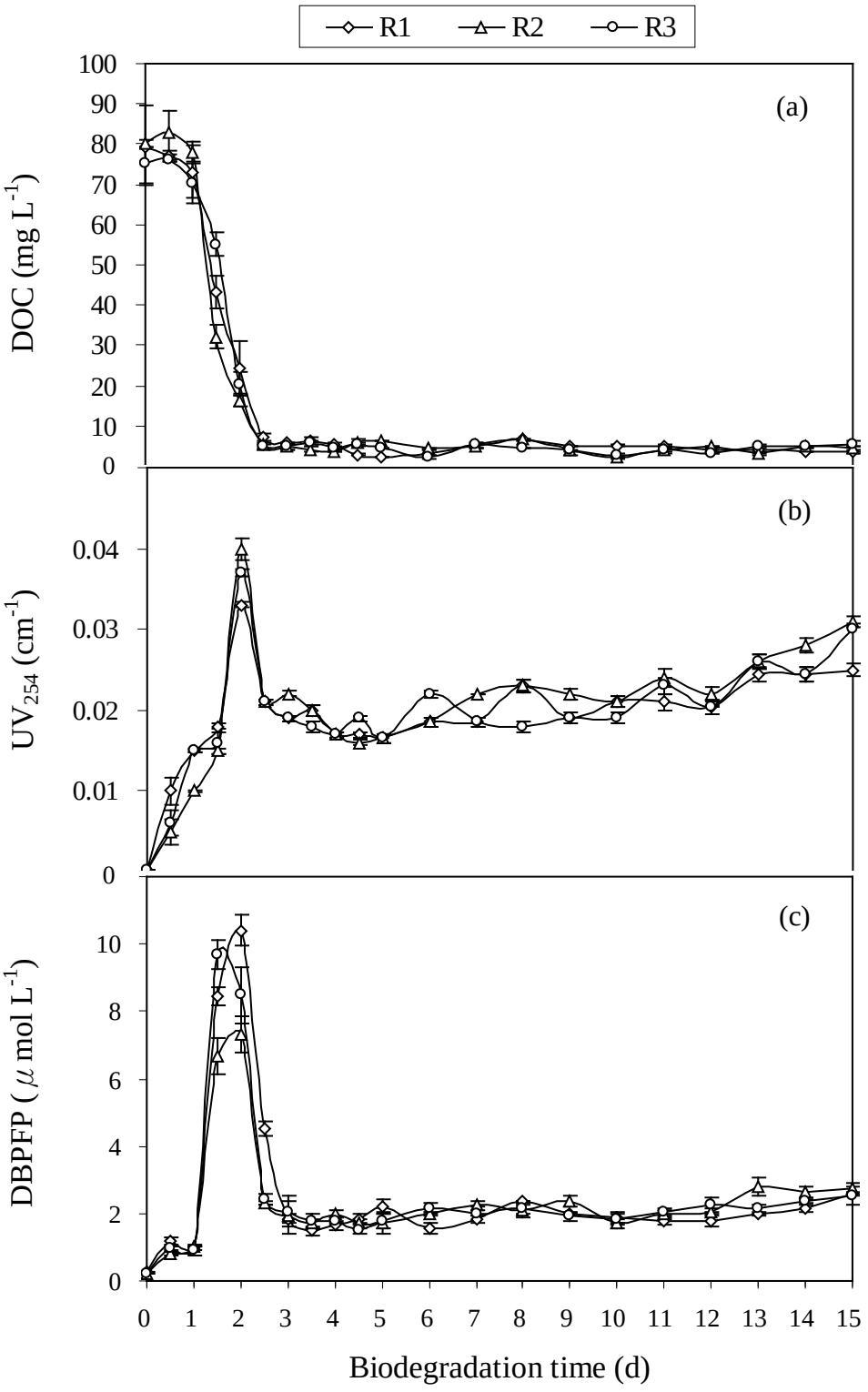

Figure S1. Changes in (a) DOC, (b) UV_{254} absorbance, and (c) DBPFP in the synthetic wastewater solutions during 15-d biodegradation incubation for three repeated experimental tests described in Section 2.1

(Repeated incubation test runs: R1 - run 1; R2 - run 2; R3 - run 3)

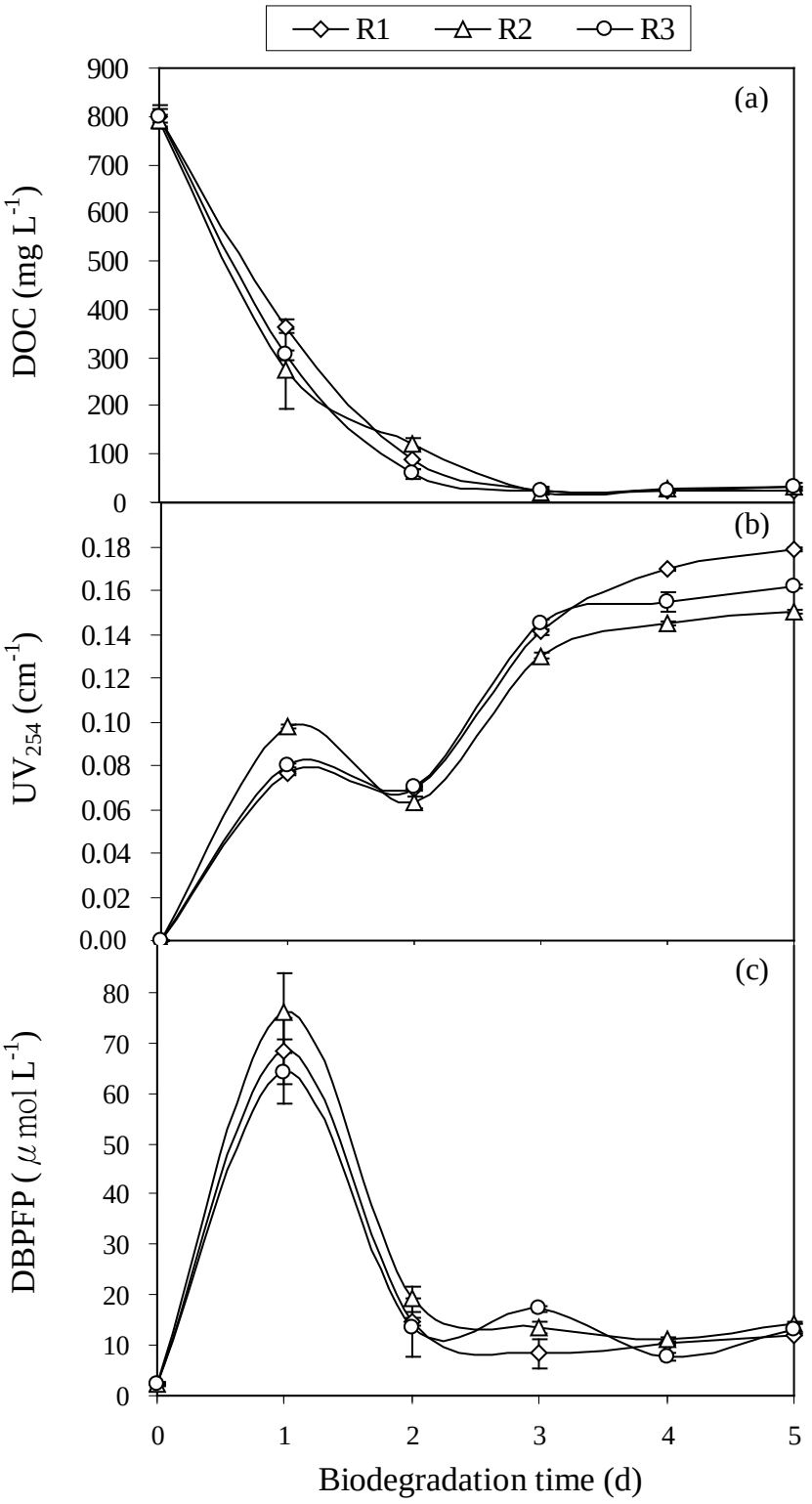

Figure S2. Changes in (a) DOC, (b) UV_{254} absorbance, and (c) DBPFP in the synthetic wastewater solutions during 5-d biodegradation incubation for three repeated experimental tests described in Section 2.2

(Repeated incubation test runs: R1 - run 1; R2 - run 2; R3 - run 3)